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AN INTRADERMAL TEST FOR BACTERIUM PULLORUM INFECTION IN FOWLS.

By ARCHIBALD R. WARD and BERNARD A. GALLAGHER, *Pathological Division.*

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DISSEMINATION OF INFECTION IN WHITE DIARRHEA.

Of the numerous diseases to which poultry are susceptible it is safe to say that bacillary white diarrhea is by far the most widespread and most destructive. Its ravages are confined principally to baby chicks, but it is the pullorum infection in the hen which is directly responsible for outbreaks of white diarrhea in the chicks, since a certain percentage of her eggs hatch infected chicks and the excretions of these spread the disease to the other birds in the brood. The exceedingly high mortality of white diarrhea, amounting in some cases to almost 100 per cent of the hatch, practically prevents the rearing of chicks in infected flocks. The disease is contracted during the first four days of life, and deaths occur as a rule during the first month. It has been demonstrated conclusively by several investigators that chicks which recover may carry the causative bacterium in the ovary and serve as a source of infection in the future. Infected hens usually exhibit an ovary containing several angular, hard, discolored ova; however, the organ may continue to functionate and from time to time an ovum is released which harbors the infective agent. Outbreaks of white diarrhea as a result of con-

NOTE.—This bulletin is a report on a study of a disease of fowls that is quite destructive, and should be serviceable to those who are interested in poultry and poultry diseases.

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taminated incubators or brooders could be controlled readily by sanitary measures, but infection through the egg must be prevented by a process of weeding out the carriers among the hens used for breeding.

THE AGGLUTINATION TEST.

Since the presence of the *Bacterium pullorum* in the ovary of the hen is not betrayed by external symptoms, it was necessary to devise a biologic method of diagnosis in order to detect the presence of the disease in the affected birds. The agglutination test was found to be applicable for this purpose, and several agricultural experiment stations have taken up the work on an extended scale, offering the service to poultrymen at a price that barely covers the cost of the work. This, in Connecticut, is understood to be 10 cents a fowl. The work of drawing blood samples and sending to a laboratory is necessarily tedious and relatively expensive as compared with the value of a bird. A simpler, cheaper, and equally accurate diagnostic method would undoubtedly contribute to greater popularity of this valuable work in disease prevention.

EXPERIMENTAL WORK.

The writers have undertaken to determine the possibility of preparing a biological product from *Bacterium pullorum* to be used for the diagnosis of the disease caused by that organism. The general idea was to develop a diagnostic method somewhat analogous to the intradermal tuberculin test, particularly as applied to fowls.

TEST OF ARTIFICIALLY INFECTED BIRDS.

Two strains of *Bacterium pullorum* were planted in 1,500 c. c. of plain bouillon in the amount of one loopful each. This culture was incubated at 37° C. from September 19 to October 19, 1914. It was then placed in the ice box until May 4, 1915. On this date 100 c. c. of the culture was passed through a Berkefeld filter. The filtrate was determined to be sterile by cultural tests. Carbolic acid was then added in sufficient quantity to make a 0.5 per cent solution.

On May 17, 1915, two drops of this filtrate were injected into the right wattle of a hen that had been injected with *Bacterium pullorum* on September 22, 1914. The liquid was injected slightly above the lower border of the wattle and no attempt was made to place it within the layers of skin. Twenty-four hours later the wattle showed an edematous swelling. The following day, 48 hours after injection, there was noted a pronounced edematous infiltration of the entire wattle. A swelling of this size in other intradermal tests would be considered as positive. The temperature was normal. On May 20 the swelling of the wattle decreased considerably, and 90 hours after

injection the wattle appeared normal. The wattle of a control, a noninfected bird, injected at the same time, remained normal. On autopsy the ovary of the infected bird presented several angular ova typical of pullorum infection. A pure culture of *B. pullorum* was isolated from the ovary. The result of this experiment suggested that a diagnostic test might be developed.

Work with the same filtrate was continued by evaporating 100 c. c. to one-tenth of its volume in a water bath at the boiling point, the purpose being to test the value of a culture filtrate containing the products of the organism in a more concentrated form.

Twelve fowls were injected intravenously on May 27, 1915, with 1.5 c. c. each of a 48-hour bouillon culture of six strains of *B. pullorum.* About two days after inoculation the fowls showed marked symptoms of illness—pale comb, drowsy attitude, and ruffled feathers. Five of this lot died within a period of 26 days as a result of the injection. On autopsy the following lesions were observed: Livers enlarged, darker than normal, and covered with necrotic foci. Spleens were enlarged and studded with necrotic foci. Ovaries contained irregular-shaped hard, dark-colored ova typical of pullorum infection. In one case there was severe pericarditis and in another considerable amber-colored fluid in the peritoneal cavity.

On June 9, thirteen days after the fowls were inoculated and while they still were sick, blood was drawn for serum tests. Of these, fowl 73 was injected in the right wattle with 0.1 c. c. of the concentrated filtrate. Three hours after injection considerable edema of the wattle was observed.

Two control fowls, 74 and 75, supposedly healthy birds, each received 0.1 c. c. of the concentrated filterate in the right wattle. Three hours later they showed edema of the wattle, practically of the same extent as that shown by the infected bird.

On the next day the swelling of the wattle of the control fowls 74 and 75 had entirely disappeared, while fowl 73 showed considerable swelling. This swelling continued to be marked at the forty-eighth hour, after which it began to subside.

The results of this test are shown in Table I and include the results of autopsy, cultural, and agglutination tests.

TABLE I.—*Concentrated filtrate tested on fowls 13 days after inoculation.*

Fowl No.—	Edema after 3 hours, June 9.	Edema after 24 hours, June 10.	Edema after 48 hours, June 11.	Autopsy.	Culture.	Agglutination, 1 : 100.
73	+	+	+	+	+	+
74 [1]	+	−	−	−	−	−
75 [1]	+	−	−	−	−	−

[1] Controls.

In this second test, while the concentrated filtrate gave satisfactory results, the reaction was not as marked as in the case of the first fowl, on which the nonconcentrated fluid was used.

On June 22 a third series of trials with the filtrate was conducted upon 6 fowls that had been inoculated intravenously on May 27 with a culture of *Bacterium pullorum.*

Fowls 77, 78, and 81, with two controls, 86 and 87, received in the right wattle 0.1 c. c. of the same concentrated filtrate as used before. In each case the injected wattles showed some edema within an hour.

On June 24 fowls 77 and 87 still showed slight edema, while fowl 81 showed a considerable swelling. The results of these tests are shown in Table II, together with data on agglutination test, autopsy, and cultures from ovaries made at a later date.

TABLE II.—*Concentrated filtrate tested on fowls 26 days after inoculation.*

Fowl No.—	Edema after 1 hour, June 22.	Edema after 24 hours, June 23.	Edema after 48 hours, June 24.	Autopsy.	Culture.	Agglutination, 1 : 100.
77	Slight...	Slight...	Slight...	+	+	+
78	...do....	—	—	+	+	+
81	...do....	Slight...	+	?	+	+
86 [1]	...do....	—	—	—	—	—
87 [1]	...do....	Slight...	Slight...	0	0	0

[1] Controls.

At the same time that the foregoing test was made a similar one was conducted with the original nonconcentrated filtrate.

Infected fowls 76, 79, and 82, together with fowls 88 and 89, supposedly noninfected controls, were injected in the right wattle with 0.1 c. c. of nonconcentrated filtrate. All showed a discernible edema shortly after injection.

On June 23 a slight edema persisted in fowls 76, 79, 82, and 88 (control). On June 24 fowl 88, the control, still showed a slight edema, fowl 76 showed a slight reaction, while fowls 79 and 82 showed swelling indicative of a fair positive reaction. The results of these tests and other data are given in Table III.

TABLE III.—*Nonconcentrated filtrate tested on fowls 26 days after inoculation.*

Fowl No.—	Edema after 1 hour, June 22.	Edema after 24 hours, June 23.	Edema after 48 hours, June 24.	Autopsy.	Culture.	Agglutination, 1:100.
76	Slight.	Slight.	Slight.	+	+	+
79	Slight.	Slight.	+	+	+	+
82	Slight.	Slight.	+	?	+	+
88 [1]	Slight.	Slight.	Slight.	+	+	+
89 [1]	Slight.	—	—	—	—	—

[1] Controls.

Comparing the results shown in Tables II and III, it would appear that the nonconcentrated filtrate has up to date given the most satisfactory results.

On August 16, 1915, tests of the original nonconcentrated filtrate were made. Fowls 73, 76, 77, 78, 79, 81, and 82 were injected with 0.1 c. c. in the left wattle. Of these, fowls 73, 81, and 82 showed reactions at 48 hours. These had reacted previously, fowl 73 on June 9, and the other two on June 22.

Fowls 76, 77, and 78 did not react from the injection on August 16, and it is noted that they had not reacted well from the injection on June 22. At that time fowl 78 was negative and fowls 76 and 77 were rated as slight. Of four controls, fowls 95, 96, 97, and 98, injected with filtrate on August 16, one, fowl 95, gave a reaction.

Table IV shows the present test, together with previous and subsequent tests on these birds. For convenience the table includes the results of two succeeding tests on some of these birds, together with the results of the agglutination test, autopsy, and cultures from ovaries made at a later date.

TABLE IV.—*Retests of fowls with various test products.*

Fowl No.—	Concentrated filtrate.		Nonconcentrated filtrate.		Concentrated filtrate.		Nonconcentrated filtrate.		Dead culture.				Autopsy.	Culture from ovary.	Agglutination, 1 : 100.
	June.		June.		June.		Aug.		Sept.						
	10	11	23	24	23	24	17	18	3	4	21	22			
73	+	+	.....	.....	.....	.....	+	+	+	+	+	+	+	+	+
76	.....	.....	Slight.	Slight.	.....	.....	−	−	.....	.....	.....	.....	+	+	+
77	.....	.....	.....	.....	Slight.	Slight.	−	−	Slight.	Slight.	Slight.	Slight.	+	+	+
78	.....	.....	.....	.....	−	−	−	−	.....	.....	+	Slight.	+	+	+
79	.....	.....	Slight.	+	.....	.....	−	−	.....	.....	+	+	+	+	+
81	.....	.....	.....	.....	Slight.	+	+	+	.....	.....	+	+	?	−	+
82	.....	.....	Slight.	+	.....	.....	+	+	.....	.....	+	Slight.	?	−	+
95[1]	.....	.....	.....	.....	.....	.....	+	+	.....	.....	.....	.....	+	+	+
96[1]	.....	.....	.....	.....	.....	.....	−	−	.....	.....	.....	.....	−	−	−
97[1]	.....	.....	.....	.....	.....	.....	−	−	.....	.....	.....	.....	−	−	−
98[1]	.....	.....	.....	.....	.....	.....	−	−	.....	.....	.....	.....	−	−	−

[1] Controls.

The fact as shown in the tables that three controls, fowls 87, 88, and 95, gave slight reactions, suggested the idea that an edema persisting for 24 hours or longer after injection might possibly be induced by causes such as irritation from the carbolic acid in the test fluid or puncture by the hypodermic needle. In order to determine this point, 6 fowls were tested, as follows: Two were injected in the wattle with 0.1 c. c. of plain bouillon, 2 with 0.1 c. c. of boullion carbolized to the same degree as the culture filtrate, and 2 received the hypodermic-needle puncture only. No reaction occurred

as a result, and there remained the alternative conclusion that the test was either nonspecific or that the reacting controls were infected. Some of the control birds had been secured in the open market, and nothing was known of their previous exposure. Unfortunately control fowl 87, which showed a slight reaction on June 23, died from intercurrent causes and in the absence of the authors was not autopsied. As appears in autopsy notes later, control fowls 88 and 95, which also had reacted, were found to be infected birds.

On August 25 fowls 76, 95, and 96 and a control supposedly negative were killed by bleeding and samples held for agglutination tests. The ovary of fowl 76 contained angular ova typical of pullorum infection, that of fowl 96 dried encapsulated ovum and cysts, not certainly due to pullorum infection, and that of fowl 95 one or two angular ova and two large cysts.

Cultures were made from ovaries of fowls 76 and 95 which yielded growths characteristic of *Bacterium pullorum*, while that from fowl 96 yielded a heavy growth not resembling that of *Bacterium pullorum*. Fowl 76 gave positive agglutination in 0.01 dilution; fowl 95 in 0.002 dilution, while fowl 96 was negative at 0.04 dilution.

An antigen was made from the culture obtained from fowl 96 and tested against the serum of fowl 76, but gave a negative agglutination at 0.04. The result further strengthens the conclusion that the infection in the ovary of fowl 96 was not due to *Bacterium pullorum*. A check was run on the serum of fowl 76 with *Bacillus abortus* as an antigen, with no agglutination resulting. It is seen that fowl 76, although it did not react well to the intradermal test, gave an agglutination of 1:100. Also, fowl 95 was undoubtedly infected with *Bacterium pullorum*.

With a view of securing a diagnostic agent that would increase the size of the swelling in the wattle, the writers next tried a product consisting of six strains of *B. pullorum* which had been grown in plain bouillon at a temperature of 37.5° C. for one month. The culture was killed by heating at 60° C. for one hour in a water bath and then carbolized to 0.5 per cent. The organisms were not removed from the medium, and this product was employed in all subsequent tests.

TABLE V.—*Tests with killed bouillon cultures.*

Fowl No.—	Edema after 3 hours, Sept. 2.	Edema after 24 hours, Sept. 3.	Edema after 48 hours, Sept. 4.	Autopsy.	Culture from ovary.	Agglutination, 1:100.
73	Marked	Marked positive	Positive	+	+	+
77	Considerable	Slight	Trace	+	+	+
186[1]	Slight	—	—	0	0	0

[1] Control.

On September 2, fowls 73 and 77 and control fowl 186 were injected with 0.1 c. c. As shown in Table V edema was present at 24 hours in fowls 73 and 77 and entirely absent in control fowl 186.

On September 20 the following birds were injected in the wattle with 0.1 c. c. of the same bacterial product as used in the preceding test: Fowls 73, 77, 78, 79, 81, 82, and controls 55, 74, 88, 89, 90, 92, 97, 98, and 99. The results of the test are shown in Table VI.

TABLE VI.—*Further tests with killed bouillon cultures.*

Fowl No.—	Edema after 24 hours, Sept. 21.	Edema after 48 hours, Sept. 22.	Autopsy.	Culture.	Agglutination, 1:100.
73	+	+	+	+	+
77	Slight...	Slight...	+	+	+
78	+	do	+	+	+
79	+	+	+	+	+
81	+	+	?	+	+
82	+	Slight...	?	−	+
55[1]	−	−	0	0	Not tested.
74[1]	−	−	−	−	−
88[1]	+	Slight...	+	+	+
89[1]	−	−	−	−	−
90[1]	−	−	0	0	Not tested.
92[1]	−	−	0	0	Do.
99[1]	+	−	−	−	−

[1] Controls.

On September 23, the following fowls were killed by bleeding and blood was saved for agglutination tests. Autopsy notes follow:

Fowl 73. A hard tumorlike mass about an inch in diameter is attached to the ovary by fibrous threads and vessels. Other small ova show typical appearance of pullorum infection. *Bacterium pullorum* was recovered in pure culture from the ovary.

Fowl 77. Ovary contains one ovum the size of a pea, having the appearance of an old pullorum ovum. *B. pullorum* was obtained in pure culture.

Fowl 78. One ovum the size of a pea and having characteristic color of those with pullorum infection. Also several smaller similar ones and a cyst the size of a hickory nut which is filled with a colorless fluid. *B. pullorum* was obtained in pure culture from the ovum.

Fowl 79. Ovary contains a number of typical pullorum ova. Pure culture of *B. pullorum* was obtained.

Fowl 81. Ovary contains large ova apparently normal. There are some small brownish ova that may or may not be infected. Liver contains a few necrotic spots. Cultures from both liver and ovum gave negtive results.

Fowl 82. Large ova apparently normal. Some small brownish ova that may or may not be infected. Liver contains a few whitish spots. Cultures from both liver and ova gave negative results.

On September 24, the following control birds were killed by bleeding, and serum was saved for agglutination tests: Fowls 86, 74, 88, 89, 97, 98, and 99. All were normal, and cultures were negative except for fowl 88, which had reacted to the wattle test. This

bird was found to possess a typical pullorum ovary, and yielded a pure culture of *Bacterium pullorum.*

In preparation for another set of trials of the test upon artificially infected fowls, on August 26, 1915, the following birds received intravenous injections of 1 c. c. of bouillon culture of nine strains of *B. pullorum* mixed: Fowls 28, 67, 85, 182, 294, 204, and 215. On September 7 the following birds were injected intraabdominally with 5 c. c. of a similar mixture of strains: Fowls 16, 18, 32, 34, 36, 37, 38, 39, 45, 46, 47, 48, 49, 64, 65, 69, 76, 178, 190, and 197. Fowls 28, 85, and 215 died shortly after the injection.

The birds were tested with killed bacterial culture. The results of the two tests, with autopsy findings and results of cultures inoculated from the ovaries, are given in Table VII.

TABLE VII.—*Two tests with nonconcentrated killed culture.*

Fowl No.—	Dec. 7–8.		Feb. 9–10.		Autopsy Mar. 16.	Cultures
16	+	+	+	−	Questionable	−
18	+	−	−	−	Positive	+
32	+	+	+	+	do	+
34	−	−	Trace.	−	do	+
36	+	+	Trace.	−	Questionable	−
37	−	−	+	−	do	−
38	+	+	+	Trace.	Positive	−
39	+	+	+	−	Normal	−
45	+	+	+	−	do	−
46	+	+	Trace.	−	do	−
47	+	+	+	+	Positive	−
48	+	+	−	−	Normal	−
49	+	+	Trace.	−	Questionable	−
64	+	+	+	−	Positive	+
65	+	−	−	−	Normal	−
67	+	+	Trace.	−	Positive	−
69	+	−	+	−	Questionable	+
176	+	+	+	−	Positive	+
178	+	−	+	Trace.	do	+
190	+	+	+	Trace.	do	−
197	+	−	−	−	do	+
182	+	+	+	Trace.	do	−
204	+	+	+	Trace.	Questionable	−
294	+	−	Trace.	−	Normal	−
41 [1]	−	−	−	−	Not killed	
42 [1]	−	−	−	−	do	
77 [1]	−	−	−	−	Normal	
278 [1]	+	−	−	−	Died	−
179 [1]	−	−	−	−	Normal	
80 [1]	−	−	−	−	Not killed	
181 [1]	−	−	−	−	do	
101 [1]	−	−	−	−	do	
102 [1]	−	−	−	−	do	
103 [1]	−	−	−	−	do	

[1] Controls.

SUMMARY OF THE TESTS WITH ARTIFICIALLY INFECTED BIRDS.

In the course of the experiments recorded in the foregoing, 32 birds that had been exposed to infection by injection of live cultures were employed. When tested for the first time 29 of these, or 90 per cent, revealed edematous swellings rated as either slight or positive at 24 hours after injection with the diagnostic agent. When read at a 48-hour interval, 23, or 71 per cent, of the same birds gave

reactions rated as either slight or positive. Thus, the 24-hour interval yielded the largest percentage of reactions. Practically all birds, both those inoculated and controls, exhibited a swelling shortly after injection and therefore no diagnostic value has been attributed to swellings observed before the lapse of 24 hours.

Three birds gave negative readings at both 24 and 48 hours. Autopsy of two of these revealed unquestionable lesions of pullorum infection, from which the organisms were obtained, while in the third one the lesions were questionable and no culture was obtained. Thus, the test failed to detect 6 per cent of the birds in which lesions were found.

In all but two cases the same birds were retested after an interval of 7 or 8 weeks. Of the 30 birds retested 22, or 73 per cent, gave a reaction rated as either a trace, slight, or positive at 24 hours on the second test. At 48 hours on the second test only 8, or 26 per cent, displayed reactions rated as a trace, slight, or positive. Further, 8 birds, or 26 per cent, showed no reaction at either 24 or 48 hours. It is evident that a retest after an interval of about 8 weeks is far less reliable than a first test.

Of the 32 birds tested, autopsy revealed unquestionable lesions in 18, or 56 per cent. In 8, or 25 per cent, the lesions were regarded as questionable. In 6 birds, or 16 per cent, no lesions were found, although all were positive to the test at 24 hours.

Twenty-six controls were tested for the first time. These had been gathered from various sources and there was no assurance that they were free from infection. Of these, 5 at 24 hours after injection displayed swellings rated as slight or positive and 4 displayed the same condition at 48 hours. At autopsy 2 were found to be infected, and 1 through accident was not examined. No lesions were found at autopsy of 2; however, 1 of these came from the same flock as one of the unquestionably infected controls, and had been in the same cage as the infected bird.

While agglutination tests were made on serum drawn from inoculated birds, after injection with the diagnostic agent, and the results appear in the various tables, it is realized that agglutination would naturally be expected as a result of the various injections. We have observed that as a result of the artificial infection with cultures of *Bacterium pullorum*, the agglutinating value of the serum of these birds varied within a wide range. Some birds gave an agglutination at a dilution of 1:1,000, while others that had been repeatedly injected with the test fluid gave no agglutination, owing to the strong bacteriolytic properties of their sera, presumably resulting from the various injections. Negative control birds after one injection with the test fluid gave an agglutination titer of 1:50.

The disadvantages of work with artificially infected birds, due to the large amounts of culture injected and to the severe reactions resulting, were thoroughly realized, and work with naturally infected birds was undertaken.

FIELD TRIALS OF THE INTRADERMAL TEST.

Through the courtesy of the Connecticut agricultural experiment station, opportunity was afforded to apply the intradermal test to two flocks tested at the same time by Dr. L. F. Rettger by the agglutination method.

One flock of 231 birds injected on February 28, 1916, contained at the time over 40 birds showing more or less evidence of swelling of the wattles due to frostbite, while 6 others showed very slight swelling attributed to the same cause. When examined 38 hours after injection none was regarded as showing reaction to the intradermal test. One bird gave a reaction to the agglutination test and was killed by the owner before arrangements were made to retest by the intradermal method. However, the owner had made an autopsy and reported that he regarded the bird as infected.

In the second flock in which work was done the Connecticut Agricultural Experiment Station tested 50 birds in the regular routine work of testing. Of these 1 reacted to the agglutination test and failed to react to the intradermal test when examined 46 hours after injection. A number of birds showed slight abnormal conditions, regarded at the time as due to frostbite, but noted in connection with the problem of determining the least amount of swelling to be regarded as a significant intradermal reaction, under the conditions in question.

The bird that gave a positive reaction to the agglutination test was retested by both methods about a month later by Dr. Rettger. At 24 hours after injection the wattle was swollen to about 2.5 times normal thickness, and when observed at 48 hours the swelling was 1.5 times normal. An agglutination test made at the same time also gave positive results. It is probable that the failure of the intradermal test when used the first time was due to some error in technique. Further, it is the belief of the writers that readings should be taken at about 24 hours, and not as late as 36 and 48 hours, as in these trials.

In the same flock the intradermal test alone was applied to about 100 birds, and those showing any enlargement of the wattle at 46 hours were tested by the agglutination method by Dr. Rettger. The results yielded by both methods are given in Table VIII. The size of the swelling following the intradermal injection is indicated as nearly as possible by arranging them in order of decreasing size from the top to the bottom of the list. Here, again, cognizance was taken

of every degree of swelling, without implying that the slighter swellings were significant.

TABLE VII.—*Comparison of intradermal and agglutination tests.*

Fowl No.—	Intradermal test.	Agglutination test.	Fowl No.—	Intradermal test.	Agglutination test.
93	Whole wattle swollen, ×3; droops.	−	66	Swelling and drooping of feathered skin at edge of wattle.	−
76	Whole wattle swollen, ×3	+	52	Trace of swelling at lower edge of wattle	−
77	Lower half swollen, ×3	+	99	Swelling possibly due to traumatism, as wattle is very blue	−
73	Swollen, ×2	−	60	Questionable swelling of feathered skin at edge of wattle	?+
72	Swollen, ×2	(?)	95	Trace of swelling on posterior half of wattle	−
96	Swollen, ×2	−			
29	Lower half swollen, ×2	−			
59	Lower half swollen, ×1.5	−			
81	Lower half swollen, ×1.5	−			
100	Lower half swollen, ×1.5	−			
97	Lower half swollen, ×1.5	−			

The results were particularly discordant in the case of fowl 93, which had been placed at the top of the list as showing the best intradermal reaction, while it failed to give a reaction to the agglutination test. In view of the discrepancy, Dr. Rettger obtained the bird in question, together with three others, for retest and autopsy. The results are shown in Table IX:

TABLE IX.—*Comparison of retests and autopsy findings.*

Fowl No.—	Intradermal test.		Agglutination test.	Condition of ovaries at autopsy.
	24 hours.	48 hours.		
93	Slight	−	−	Normal.
72	Swollen, ×2	Swollen ×1.5	−	Do.
29	−	−	−	Normal (small).
60	−	−	−	Normal.

The result of the retest and autopsy of birds 93 and 72 is not wholly satisfactory. The repetition of the reaction in both cases is significant; but, on the other hand, the results of the agglutination test and autopsy leaves the matter inconclusive. As to the remaining discrepancies in Table VIII, the many other cases noted as surely the result of freezing indicate that it is not desirable to apply the intradermal test where there is a possibility of freezing.

TRIALS BY OTHER INDIVIDUALS.

In several instances the test product was sent to interested individuals on request. One report on the results was received in which 1,301 birds were tested and 78 gave a positive reaction. The latter were retested by the agglutination method, and 70 gave a positive reaction.

COMPARISON OF RESULTS OF AGGLUTINATION AND INTRADERMAL TESTS ON NATURALLY INFECTED BIRDS.

Through the assistance of Roy E. Jones, we located and purchased 47 birds that had given positive or questionable agglutination tests, applied by the Connecticut Agricultural Experiment Station. These, together with nine controls, were injected for the intradermal test on June 23, 1916, and readings were taken at 24 and 48 hours.

Of the birds reported positive to the agglutination test applied by the Connecticut station, there was total agreement in 28, or 70 per cent, of the cases in that they also gave positive intradermal test as determined 24 hours after injection and displayed unquestionable lesions when eventually slaughtered. Of those reported positive to the agglutination test, 30, or 75 per cent, revealed lesions at autopsy.

Thirty-five birds gave positive reactions to the intradermal test. Autopsy revealed that of these 29, or 83 per cent, possessed undoubted lesions, in 5 the lesions were questionable, and in 1 no lesions occurred. Of those reported positive to the agglutination test, 3 birds, or 7 per cent, failed to react to the intradermal test, and autopsy revealed no lesions. On the other hand, 2 birds, or 5 per cent, that had given positive agglutination tests, gave negative intradermal tests, and autopsy revealed lesions. Thus, the percentage of absolute failures of each test as judged by the other test and by the autopsy findings were very similar in amount.

Seven birds had given questionable agglutination tests. Of these, 3 were negative to the intradermal test and negative at autopsy. One reported questionable gave a positive intradermal reaction and autopsy revealed lesions. The intradermal test on the other 3 yielded positive, negative, and questionable results, respectively, and autopsy of all 3 furnished inconclusive information.

Of the nine controls, one displayed a marked reaction at 24 hours, consisting of a swelling of the wattle to three times its normal thickness. Autopsy revealed undoubted lesions, and a pure culture of *Bacterium pullorum* was isolated from the ovary. Four others displayed traces consisting of swelling of the lower border of the wattle to about twice the normal thickness. On autopsy, one of these was found to contain undoubted lesions and a pure culture of *B. pullorum* was obtained.

The examination of the wattles at 48 hours revealed swellings varying from a trace to positive in only 22 birds, or 46 per cent, of those tested. This result compared with the 28 birds regarded as positive at 24 hours and verified by subsequent autopsy, again indicates that 48 hours is too long to secure all the positive reactions. Among the controls only 1 displayed any swelling whatsoever, and this case proved on autopsy to be positive.

On June 26 all the 47 birds and 9 controls were reinjected and examined 5 hours later. At this time every bird, including controls, displayed a swelling varying in the different individuals from a trace to five times the normal thickness. The observation merely emphasizes the fact of the occurrence of a nonsignificant swelling following injection with the diagnostic agent.

At 24 hours 39 birds displayed swelling of the wattle varying from a trace to enlargement to five times the normal thickness. Autopsy revealed undoubted lesions in 30 of these, questionable lesions in 7, and no lesions in 2. Total agreement between the results of this reading, the agglutination test, and autopsy findings occurred in 70 per cent of the birds tested. In two cases, or 4 per cent of the birds, the positive readings by the agglutination test were not supported by the negative results of the intradermal test and the autopsy. In 1 case, or 2 per cent, negative results of the intradermal test were contradicted by the positive results of agglutination test and autopsy. Thus, the results yielded by the first and second 24-hour readings of the test on supposedly infected birds vary but little.

The results yielded by the test on the control birds were perfect, as confirmed by the autopsy. The only two birds that displayed traces of swelling proved on autopsy to be infected.

The fact that the results of the agglutination test, intradermal test, and autopsy are in complete agreement in 70 per cent of the cases, coupled with the fact that the absolute diagreements are very small, indicates that the two tests are equally accurate.

The results obtained at the autopsy of the birds emphasize the difficulty of determining a standard for comparison of the accuracy of the two tests under trial. Thirty-one cases, or 64 per cent, were found to possess unquestionable lesions consisting of the angular ova characteristic of the infection. All of the cases had given positive reactions to one or both tests. In nine cases, or 10 per cent, the autopsy was inconclusive in that there were present only very small dark ova or cysts. Of these 9 questionable cases 3 had given questionable agglutination readings but positive intradermal reactions. In two cases the agglutination and intradermal tests disagreed. In four cases both tests had given positive results.

SIGNIFICANCE OF SWELLING AS AN INDICATION OF A REACTION.

In determining the significance in diagnosis of an edematous swelling of a wattle one is confronted with the fact that in all birds such swelling occurs shortly after injection. The problem is to determine the point of time after injection to read the test when this preliminary swelling has disappeared, yet not too late to escape

observing a significant edema. The tests on birds in the laboratory and probably also those in the field indicate that 48 hours is too late. While some observations on birds in the field made during freezing weather would indicate that slight swellings should not be considered, yet the entire experience with birds in the laboratory indicates that even a trace may be indicative of a positive reaction. Some few cases would indicate that a 24-hour reading might give false results due to the inclusion of some cases in which the preliminary nonsignificant swelling had not quite subsided. At present, the 24-hour interval has given the best results, but the examination of a series of readings at 30 hours would be desirable.

VARIOUS BIOLOGIC TESTS.

During the course of these experiments several attempts were made to produce a reaction to the diagnostic agent by injection into the comb, but no satisfactory results were obtained. The ophthalmic, palpebral, and subcutaneous tests also failed to produce a reaction. Also limited complement-fixation tests on the blood serum of infected fowls gave uncertain readings.

SUMMARY AND CONCLUSIONS.

A killed culture of *Bacterium pullorum* grown for about a month and held for several weeks before use and without further treatment other than carbolizing, has given the most satisfactory results.

It seems to be a fact that the edematous swelling resulting from the injection of this product into the wattle of a fowl, when observed at a proper time interval, is an indication of the presence of infection of *B. pullorum* in the fowl.

Our experience to date with readings at various time intervals leads to the conclusion that the 24-hour interval has given the most accurate results. However, it seems desirable to test on a large number of birds the accuracy of readings made at a slightly longer interval.

The weight of evidence indicates that any perceptible swelling of the wattle should be regarded as significant. A second intradermal test made at an interval of four days gave results varying but little from the first test. Others made at intervals up to two months gave less accurate results the second time. Thus, there is no advantage in retesting.

Of birds artificially infected with the disease and tested in the laboratory, in round numbers 90 per cent gave positive reactions; and in 6 per cent the test failed to indicate a reaction when lesions were present. In 3 per cent no reaction occurred and no lesions were present.

In a field test on 231 birds made simultaneously with the agglutination test, the intradermal test at 38 hours failed to detect one case reported positive to the other test. In a second flock of 50 birds in which the two tests were compared, the intradermal test when read at 46 hours failed to indicate one case that was detected by the agglutination test. Another group of about 100 birds tested under unfavorable conditions gave less satisfactory results.

Forty-seven birds that had been tested by the agglutination method by the Connecticut Agricultural Experiment Station in the field were purchased for experiments with the intradermal test. Of these, 40 had given positive reactions to the agglutination test and 7 doubtful reactions. There was complete agreement between the agglutination test, the intradermal test, and autopsy findings in 70 per cent of the cases. The agglutination test reported positive in 3 cases, or 7 per cent, was not confirmed by the intradermal test nor by the autopsy findings. The result of the intradermal test was negative in 2 cases, or 5 per cent, when it was not confirmed by the positive agglutination test and autopsy findings. Thus the percentage of absolute failures of each test was small and very similar for both tests.

Autopsy does not furnish an absolute standard for comparing the accuracy of tests. Seventy-two per cent only of naturally infected birds that had reacted to one or both tests were found on autopsy to be unmistakably infected.

The intradermal test detected the presence of infection in 4 of the 34 control birds injected in connection with the tests in the laboratory on artificially and naturally infected birds.

In a field trial not made by the writers, 1,301 birds were tested intradermally and 78 reacted. Of these 70 reacted to the agglutination tests made subsequently.

The intradermal test has already shown sufficient promise to warrant further extensive trials in the field in comparison with the agglutination test.

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